

WHAT IS CLAIMED IS:

1. A flavivirus having a phenotype in which the viral genome is modified by the introduction of a mutation, singly or in combination, taken from the group consisting of the mutations of any of Table 1-37, preferably Table 37.
2. The flavivirus of claim 1, further comprising the  $\Delta 30$  mutation.
3. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 1.
4. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 2.
5. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 3.
6. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 4.
7. The flavivirus of claim 1, wherein the flavivirus is a chimeric virus.
8. The chimeric virus of claim 7 having a dengue 1 backbone.
9. The chimeric virus of claim 7 having a dengue 2 backbone.
10. The chimeric virus of claim 7 having a dengue 3 backbone.
11. The chimeric virus of claim 7 having a dengue 4 backbone.
12. The flavivirus of claim 1, wherein the phenotype is temperature sensitivity in Vero cells or the human liver cell line HuH-7.
13. The flavivirus of claim 1, wherein the phenotype is host-cell restriction in mosquito cells or the human liver cell line HuH-7.
14. The flavivirus of claim 1, wherein the phenotype is host-cell adaptation for improved replication in Vero cells.
15. The flavivirus of claim 1, wherein the phenotype is attenuation in mice.
16. A pharmaceutical composition comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.
17. A kit comprising a pharmaceutical composition according to claim 16 in a pack or dispenser device and instructions for administration.
18. A method of producing neutralizing antibodies against dengue virus comprising the administration of a therapeutically effective amount of a pharmaceutical composition comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

19. The method of claim 18, wherein administration is by subcutaneous, intradermal, or intramuscular injection.

20. A tetravalent vaccine comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

21. An live attenuated vaccine comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

22. The live attenuated vaccine of claim 21 in unit dosage form having from about  $10^2$ – $10^9$  plaque forming units (PFU)/ml.

23. An inactivated vaccine comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

24. The inactivated vaccine of claim 23 in unit dosage form having from about 0.1 to 50 µg of E protein/ml.

25. A cDNA molecule encoding a flavivirus according to any of claims 1-15.

26. An RNA molecule encoding a flavivirus according to any of claims 1-15.

27. A method of preparing a flavivirus comprising (a) synthesizing full-length viral genomic RNA in vitro using a cDNA molecule that encodes a flavivirus according to any of claims 1-15; (b) transfecting cultured cells with the viral genomic RNA to produce virus; and (c) isolating the virus from the cultured cells.

28. A method of making a pharmaceutical composition comprising combining a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

29. A method of identifying a mutation that restricts replication in human liver cells comprising (a) introducing mutations into a dengue virus genome to make mutant viruses; (b) screening the mutant viruses for a phenotype characterized by host-cell restriction in human liver cells; and (c) determining the genetic basis for the phenotype by direct sequence analysis of the virus genome.

30. A method of identifying a mutation that promotes growth in Vero cells comprising (a) introducing mutations into a dengue virus genome to make mutant viruses; (b) screening the mutant viruses for a phenotype characterized by host-cell adaptation for improved replication in Vero cells; and (c) determining the genetic basis for the phenotype by direct sequence analysis of the virus genome.

31. A method of assembling a menu of mutations for use in fine-tuning the attenuation and growth characteristics of recombinant dengue viruses comprising (a) introducing mutations into a dengue virus genome to make mutant viruses; (b) screening the mutant viruses for a phenotype characterized by temperature sensitivity in Vero cells or human liver cells, host cell restriction in mosquito cells or human liver cells, host-cell adaptation for improved replication in Vero cells, or attenuation in mice; (c) determining the genetic basis for the phenotype by direct sequence analysis of the virus genome; and (d) performing multiple iterations of steps (a) – (c), whereby a menu of mutations is assembled.

32. The method of any of claims 29-30 further comprising introducing the mutation identified by said method into a recombinant dengue virus, and characterizing the resulting phenotype.